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(54) **METHODS FOR STORING RED BLOOD CELL PRODUCTS**

(71) Applicant: **Fenwal, Inc.**, Lake Zurich, IL (US)

(72) Inventors: **Kyungyoon Min**, Kildeer, IL (US);
Katherine Radwanski, Des Plaines, IL (US)

(73) Assignee: **Fenwal, Inc.**, Lake Zurich, IL (US)

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CPC **B01D 61/32** (2013.01); **A01N 1/0226** (2013.01); **A01N 1/0273** (2013.01); **A61M 1/0236** (2014.02); **A61M 1/3693** (2013.01); **B01D 61/28** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,766,907 A	10/1956	Wallace, Jr.
3,221,741 A	12/1965	LeVeen
3,692,493 A	9/1972	Terasaki
4,035,304 A	7/1977	Watanabe
4,066,556 A	1/1978	Vaillancourt
4,073,723 A	2/1978	Swank et al.
4,092,246 A	5/1978	Kummer
4,144,165 A	3/1979	Matz
4,162,676 A	7/1979	Talcott
4,235,233 A	11/1980	Mouwen
4,267,269 A	5/1981	Grode et al.
4,356,172 A	10/1982	Nakao et al.
4,396,383 A	8/1983	Hart
4,437,472 A	3/1984	Naftulin

4,458,733 A	7/1984	Lyons
4,467,588 A	8/1984	Carveth
4,484,920 A	11/1984	Kaufman et al.
4,507,114 A	3/1985	Bohman et al.
4,572,899 A	2/1986	Walker et al.
4,585,735 A	4/1986	Meryman et al.
4,608,043 A	8/1986	Larkin
4,776,455 A	10/1988	Anderson et al.
4,834,743 A	5/1989	Valerio
4,880,425 A	11/1989	Kuhlemann et al.
4,880,786 A	11/1989	Sasakawa et al.
4,900,321 A	2/1990	Kaufman et al.
4,961,928 A	10/1990	Holme et al.
4,976,707 A	12/1990	Bodicky et al.
4,980,277 A	12/1990	Junnilla et al.
4,997,083 A	3/1991	Loretto et al.
5,024,536 A	6/1991	Hill
5,049,146 A	9/1991	Bringham et al.
5,080,747 A	1/1992	Veix
5,100,401 A	3/1992	Patel
5,147,776 A	9/1992	Koerner, Jr.
5,176,634 A	1/1993	Smith et al.
5,248,506 A	9/1993	Holme et al.
5,250,303 A	10/1993	Meryman et al.
5,267,646 A	12/1993	Inoue et al.
5,288,605 A	2/1994	Lin et al.
5,354,262 A	10/1994	Boehringer et al.
5,364,756 A	11/1994	Livesey et al.
5,373,966 A	12/1994	O'Reilly et al.
5,387,187 A	2/1995	Fell et al.

(Continued)

FOREIGN PATENT DOCUMENTS

DE	4002693	3/1991
EP	0044864	8/1981

(Continued)

OTHER PUBLICATIONS

Shaw, DJ, "The Colloidal State", in Introduction to colloid and surface chemistry, 4th Ed., Butterworth-Heinemann. Boston, Chapter 1, pp. 1-17, (1992).*

Supplementary European Search Report and Annex for EP Application 10825379, dated Mar. 14, 2013.

Pearl, Toy, et al., Transfusion-related Acute Lung Injury: Definition and Review, Critical Care Medicine, Apr. 1, 2005, pp. 721-726, vol. 33, No. 4.

Biffl, Walter L. M.D., et al., Plasma from Aged Stored Red Blood Cells Delays Neutrophil Apoptosis and Primes for Cytotoxicity: Abrogation by Poststorage Washing but Not Prestorage Leukoreduction, Journal of Trauma—Injury Infection and Critical Care, Mar. 1, 2001, pp. 1-1, XP55055440, retrieved from the Internet: URL: <http://journals.lww.com/jtrauma/pages/articleviewer.aspx?year=2001&issue=03000&article=00005&type=abstract> [retrieved on Mar. 6, 2013].

(Continued)

Primary Examiner — Ralph Gitomer

(74) *Attorney, Agent, or Firm* — Cook Alex Ltd.

(57)

ABSTRACT

Methods and systems for processing and conditioning red blood cells are disclosed. The methods and systems may be used to make a readily transfusable red blood cell product with reduced plasma. In general, the plasma content of the supernatant of the red blood cell product is no greater than about 15%. The red blood cell products are prepared using the disclosed methods and systems remain transfusable for up to 42 days.

12 Claims, 5 Drawing Sheets

(56)

References Cited

U.S. PATENT DOCUMENTS

5,405,742 A 4/1995 Taylor
 5,423,421 A 6/1995 Inoue et al.
 5,439,882 A 8/1995 Feola et al.
 5,445,629 A 8/1995 Debrauwere et al.
 5,446,030 A 8/1995 Weisz et al.
 5,459,030 A 10/1995 Lin et al.
 5,462,526 A 10/1995 Barney et al.
 5,480,773 A 1/1996 Ogata et al.
 5,494,590 A 2/1996 Smith
 5,496,821 A 3/1996 Arduino
 5,507,525 A 4/1996 Leuenberger
 5,514,106 A 5/1996 D'Silva
 5,543,062 A 8/1996 Nishimura
 5,554,527 A 9/1996 Fickenschner
 5,560,403 A 10/1996 Balteau et al.
 5,562,836 A 10/1996 Joie et al.
 5,601,972 A 2/1997 Merryman
 5,610,170 A 3/1997 Inoue et al.
 5,624,794 A 4/1997 Bitensky et al.
 5,629,145 A 5/1997 Meryman
 5,674,741 A 10/1997 Watanabe et al.
 5,695,489 A 12/1997 Japuntich
 5,724,988 A 3/1998 Dennehey et al.
 5,769,839 A 6/1998 Carmen et al.
 5,772,644 A 6/1998 Bark et al.
 5,772,880 A 6/1998 Lynn et al.
 5,783,093 A 7/1998 Holme
 5,785,700 A 7/1998 Olson
 5,789,151 A 8/1998 Bitensky et al.
 5,792,133 A 8/1998 Rochat
 5,824,216 A 10/1998 Joie et al.
 5,827,643 A 10/1998 Conrad et al.
 5,840,252 A 11/1998 Giertych
 5,843,049 A 12/1998 Heilmann et al.
 5,858,015 A 1/1999 Fini
 5,888,824 A 3/1999 Isogowa et al.
 5,899,874 A 5/1999 Jonsson
 5,906,915 A 5/1999 Payrat et al.
 5,908,742 A 6/1999 Lin
 5,910,138 A 6/1999 Sperko et al.
 5,928,213 A 7/1999 Barney et al.
 6,039,711 A 3/2000 Headley et al.
 6,068,970 A 5/2000 Hosono et al.
 6,150,085 A 11/2000 Hess et al.
 6,251,580 B1 6/2001 Lin et al.
 6,277,556 B1 8/2001 Grode et al.
 6,348,156 B1 2/2002 Vishnoi et al.
 6,364,864 B1 4/2002 Mohiuddin et al.
 6,440,372 B1 * 8/2002 Pages 422/534
 6,447,987 B1 9/2002 Hess et al.
 6,527,957 B1 3/2003 Deniega et al.
 6,548,241 B1 4/2003 McBurney et al.
 6,566,046 B2 5/2003 Lin et al.
 6,579,672 B1 6/2003 Granger et al.
 6,652,475 B1 11/2003 Sahines et al.
 6,811,778 B2 11/2004 Page et al.
 6,857,191 B2 2/2005 Whited
 6,866,992 B2 3/2005 Lin et al.
 6,936,413 B1 8/2005 Bischof et al.
 7,011,761 B2 3/2006 Muller
 7,087,177 B2 8/2006 Min et al.
 7,156,240 B2 1/2007 Oishi et al.
 7,264,608 B2 9/2007 Bischof et al.
 7,267,817 B2 9/2007 Page et al.
 7,297,272 B2 11/2007 Min et al.
 7,332,125 B2 2/2008 Cianci et al.
 7,531,098 B2 5/2009 Robinson et al.
 2001/0049089 A1 12/2001 Dottori et al.
 2002/0131958 A1 9/2002 Chapman et al.
 2002/0146400 A1 10/2002 Cincotta
 2002/0164795 A1 11/2002 Gen
 2002/0177116 A1 11/2002 Wiggins et al.
 2003/0113704 A1 6/2003 Stassinopoulos
 2003/0124504 A1 7/2003 Bitensky et al.
 2003/0148256 A1 8/2003 Payrat et al.

2003/0153074 A1 8/2003 Bitensky et al.
 2004/0029096 A1 2/2004 Steen
 2004/0043374 A1 3/2004 DePablo et al.
 2004/0106094 A1 6/2004 Payrat et al.
 2004/0132207 A1 7/2004 Arima et al.
 2004/0137417 A1 7/2004 Ryan
 2005/0031596 A1 2/2005 Crowe et al.
 2005/0053516 A1 3/2005 Whitaker et al.
 2005/0064381 A1 3/2005 Lucas et al.
 2005/0074743 A1 4/2005 Purmal et al.
 2005/0137516 A1 6/2005 Min et al.
 2005/0137517 A1 6/2005 Blickham et al.
 2005/0208462 A1 9/2005 Bitensky et al.
 2005/0233302 A1 10/2005 Hess et al.
 2005/0256443 A1 11/2005 Bischof et al.
 2005/0277108 A1 12/2005 Bitensky et al.
 2006/0127375 A1 6/2006 Livesey
 2006/0226090 A1 10/2006 Robinson et al.
 2006/0275271 A1 12/2006 Chow
 2006/0292544 A1 12/2006 Hssanein et al.
 2007/0020607 A1 1/2007 Meryman
 2007/0048726 A1 3/2007 Baust et al.
 2007/0105220 A1 5/2007 Crowe et al.
 2007/0178168 A1 8/2007 Ho et al.
 2007/0178434 A1 8/2007 Natan et al.
 2007/0190636 A1 8/2007 Hassanein et al.
 2007/0298406 A1 12/2007 Martorell Pena et al.
 2008/0050275 A1 2/2008 Bischof et al.
 2008/0176209 A1 7/2008 Muller et al.
 2008/0233554 A1 9/2008 Sehgal et al.
 2009/0239208 A1 * 9/2009 Mayaudon et al. 435/2
 2013/0203042 A1 8/2013 Min et al.

FOREIGN PATENT DOCUMENTS

EP 0301250 A1 1/1989
 EP 0367271 A1 9/1990
 EP 0526678 10/1993
 EP 526678 10/1993
 EP 0875756 A2 11/1998
 WO 8803027 A1 5/1988
 WO 9214360 A1 9/1992
 WO WO-93/01858 2/1993
 WO 9416099 A1 7/1994
 WO 9629864 A1 10/1996
 WO WO 96/40857 12/1996
 WO 9716967 A1 5/1997
 WO 0223988 A1 3/2002
 WO 2004105483 A1 12/2004
 WO 2006088455 A1 8/2006
 WO 2007054160 A2 5/2007
 WO 2007082916 A1 7/2007
 WO 2008037481 A2 4/2008
 WO 2008089337 A1 7/2008
 WO 2008107724 A2 9/2008
 WO 2008113017 A2 9/2008

OTHER PUBLICATIONS

Kelher, M.R., et al., Plasma from Stored Packed Red Blood Cells and MHC Class I Antibodies Cause Acute Lung Injury in a 2-event In Vivo Rat Model, Blood, Feb. 26, 2009, pp. 2079-2087, vol. 113, No. 9.
 Silbert, J.A., The Storage of Washed Human Erythrocytes in a Protein-Free Medium, Transfusion, Jan. 1, 1983, pp. 8-10.
 Hogman et al., Sep. 2006, Storage of Red Blood Cells with Improved Maintenance of 2, 3 Biphosphoglycerate, Transfusion, vol. 46, pp. 1543-1552.
 Heaton, 1992, Evaluation of Posttransfusion Recovery and Survival of Transfused Red Cells, Transfusion Medicine Reviews, vol. 6, pp. 153-169.
 Hess et al., 2002, Storage of Red Blood Cells; New Approaches, Transfusion Medicine Reviews, vol. 16, pp. 283-295.
 Hogman et al., 2002, Improved Maintenance of 2,3-DPG and ATP in RBCs Stored in a Modified Additive Solution, Transfusion, vol. 42, pp. 824-829.
 Tinmouth et al., 2001, The Clinical Consequences of the Red Cell Storage Lesion, Transfusion Medicine Reviews, vol. 15, pp. 91-107.

(56)

References Cited**OTHER PUBLICATIONS**

Hogman et al., 1999, Storage Parameters Affecting Red Blood Cell Survival and Function After Transfusion, *Transfusion Medicine Reviews*, vol. 13, pp. 275-296.

Hess et al., The Effect of Two Additive Solutions on the Posthaw Storage of RBCs, *Transfusion*, Jul. 2001, pp. 923-927, vol. 41.

Hess et al., Twelve-week RBC Storage, *Transfusion*, Jul. 2003, pp. 867-872, vol. 43.

Hogman et al., "Clinical usefulness of red cells preserved in protein-poor mediums", *New England Journal of Medicine*, 1978, vol. 229 (25), pp. 1377-1382.

Hogman et al., "Storage of whole blood before separation: the effect of temperature on red cell 2, 3 DPG and the accumulation of lactate", *Transfusion*, 1999, vol. 39 (5) pp. 492-497.

D. Mazar, A. Dvilansky and N. Meyerstein; Prolonged Storage of Red Cells: The Effect of pH, Adenine and Phosphate; *Vox Sang* 1994; 66:264-269; Hematology Service and the Dr. Joseph Kaufmann Hematology Laboratory, Ben Gurion University of the Negev, Beer-Sheva, Israel.

John R. Hess, Neeta Rugg; Jenny K. Gormas, Amy D. Knapp, Heather R. Hill, Cynthia K. Oliver, Lloyd E. Lippert, Edward B. Silberstein and Tibor J. Greenwalt; RBC Storage for 11 Weeks; *Transfusion* 1586-1590; vol. 41, Dec. 2001; Blood Components.

WebMD, Phosphate in Blood, last viewed on Nov. 15, 2011 at <http://www.webmd.com/a-to-z-guides/phosphate-in-blood>.

E. Beutler and W. Kuhl; Volume Control of Erythrocytes During Storage, The Role of Mannitol; pp. 353-357; XP-000909765; Department of Basic and Clinical Research, Research Institute of Scripps Clinic, LaJolla, California (1988).

European Search Report and Annex for EP Application 09004074, dated Feb. 26, 2010; pp. 1-3; Munich, Germany.

Hess et al., "Alkaline CPD and the preservation of RBC 2,3-DPG", *Transfusion*, 2002, vol. 42, pp. 747-752.

Bohmer et al., "The effect of stress upon hydrolysis adenine nucleotides in blood serum of rats", *Pharmacology, Biochemistry and Behavior*, (2003) vol. 75, pp. 467-471.

Graefe, et al., "Sensitive and Specific Photometric Determination of Mannitol in Human Serum", *Clinic Chem lab Med.*, (2003), vol. 41(8), pp. 1049-1055.

Jacobs, et al, Determination of Citric Acid in Serum and urine Using Br82, *Journal of Nuclear Medicine*, (1964), vol. 5, pp. 297-301.

MedlinePlus, Glucose test—blood, last viewed on Nov. 15, 2011 at <http://www.nlm.nih.gov/medlineplus/ency/article/003482.htm>.

Common Laboratory (LAB) Values—ABGs—Arterial blood gases, last viewed on Nov. 15, 2011 at http://www.globalrph.com/abg_analysis.htm.

Hess, J.R., "An update on solutions for red cell storage", *Vox Sanguinis*, (2006) vol. 91 pp. 13-19.

Pietersz, et al., "Platelet Concentrates Stored in Plasma for 72 hours at 22 C Prepared from Buffycoats of Citrate-Phosphate-Dextrose Blood Collected in a Quadruple-Bag Saline-Adenine-Glucose-Mannitol System", *Vox Sang.*, (1985), vol. 49, pp. 81-85.

Extended European Search Report and Office Action received from the European Patent Office for EP Application No. 09004074.2 dated Mar. 5, 2010.

Notice of Transmittal of International Search Report & Written Opinion received from the International Searching Authority for PCT/US10/50036 dated Jan. 21, 2011.

EP Communication dated May 29, 2012 for EP Application No. 12000538 with European Search Report and Annex dated May 21, 2012.

Notification of Transmittal of International Preliminary Report on Patentability for PCT/US2010/050036 dated May 3, 2012.

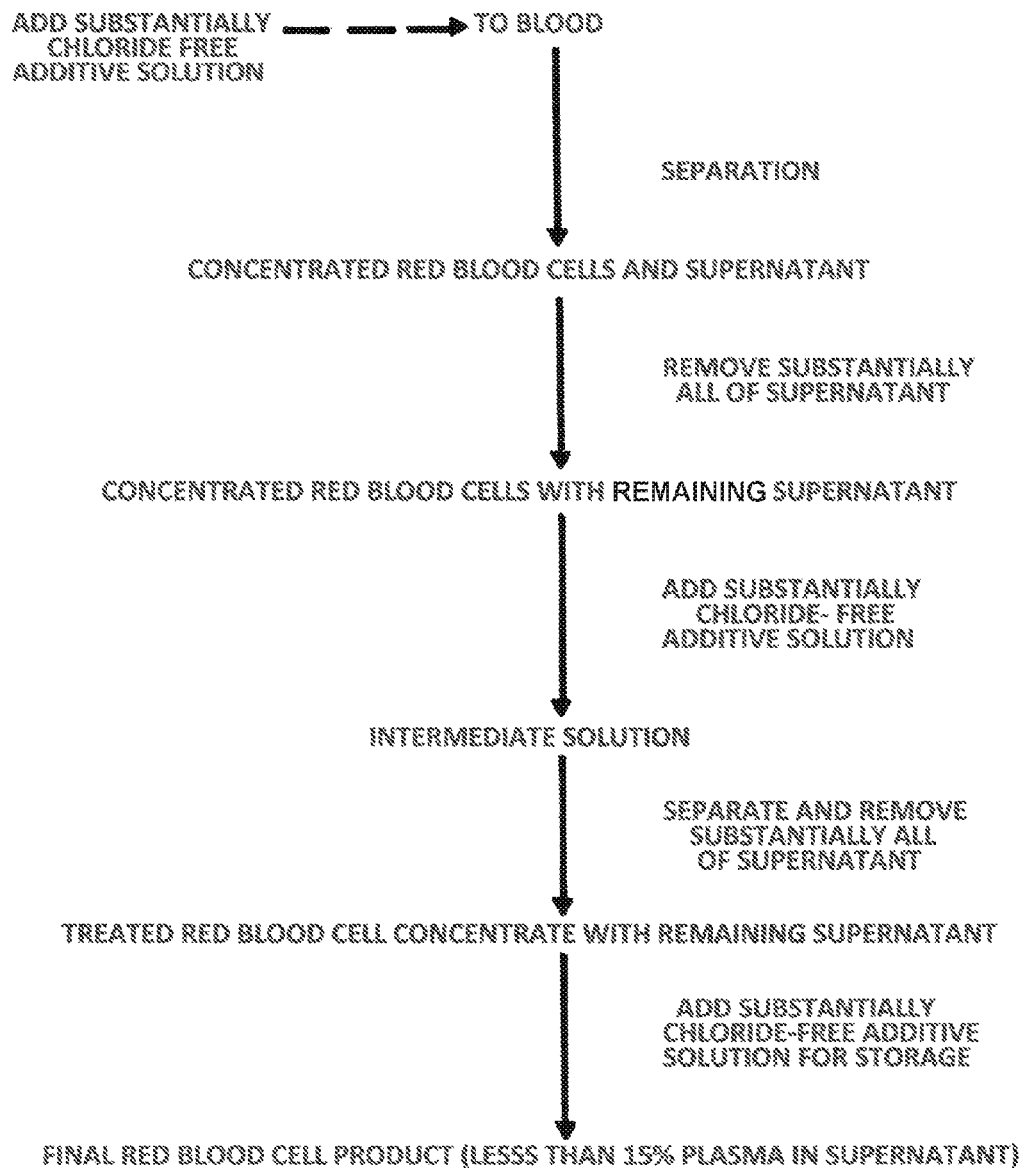
Office Action from the United States Patent and Trademark Office dated Jul. 24, 2015 in U.S. Appl. No. 13/836,829.

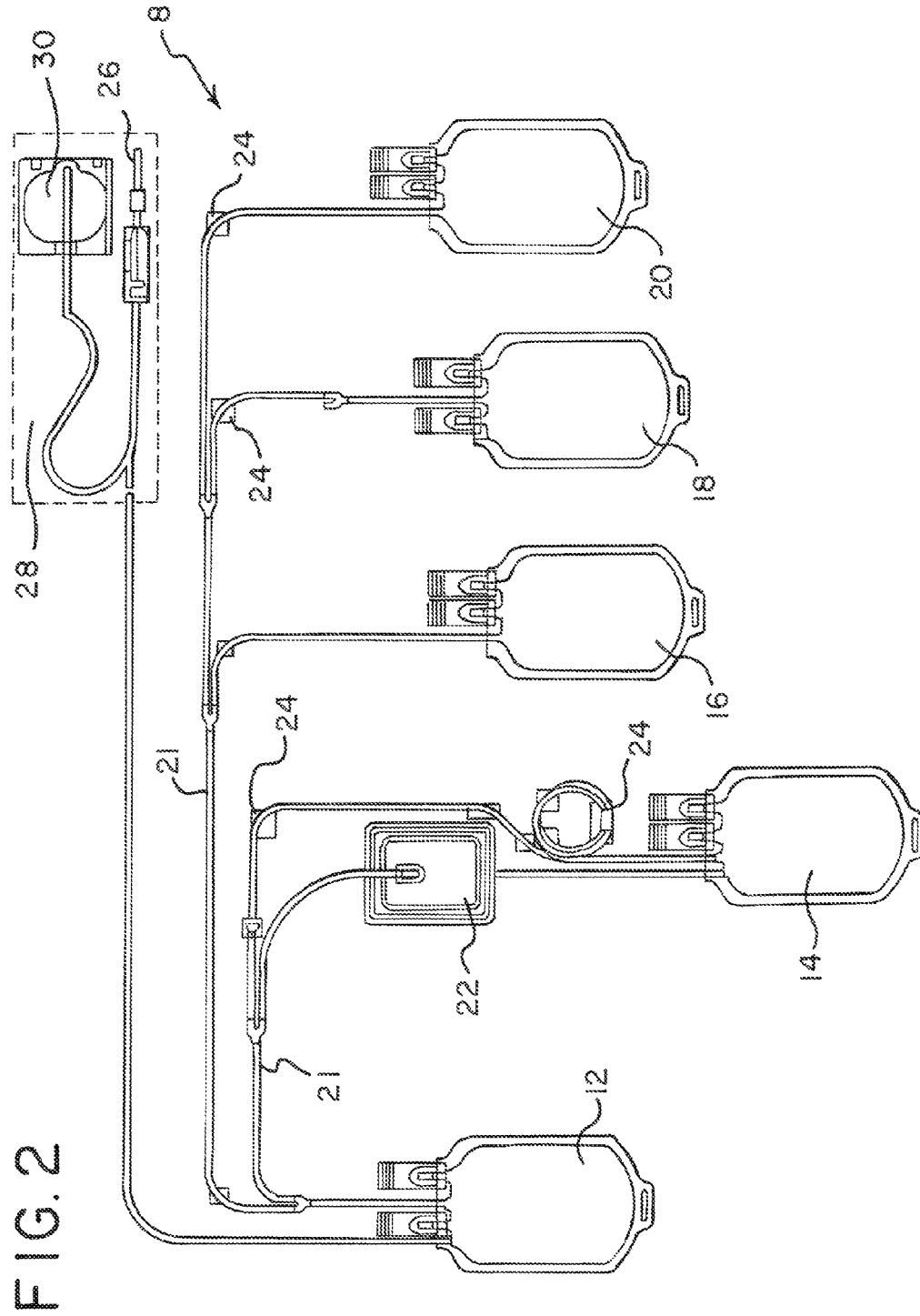
Extended European Search Report from the European Patent Office dated Jul. 28, 2014, in European Application No. 14160035.3-1651.

Edward L. Snyder, et al., entitled Collection of Two Units of Leukoreduced RBC's From a Single Donation . . . System, vol. 43, Dec. 2003, *Transfusion*, pp. 1695-1705.

* cited by examiner

FIG. 1





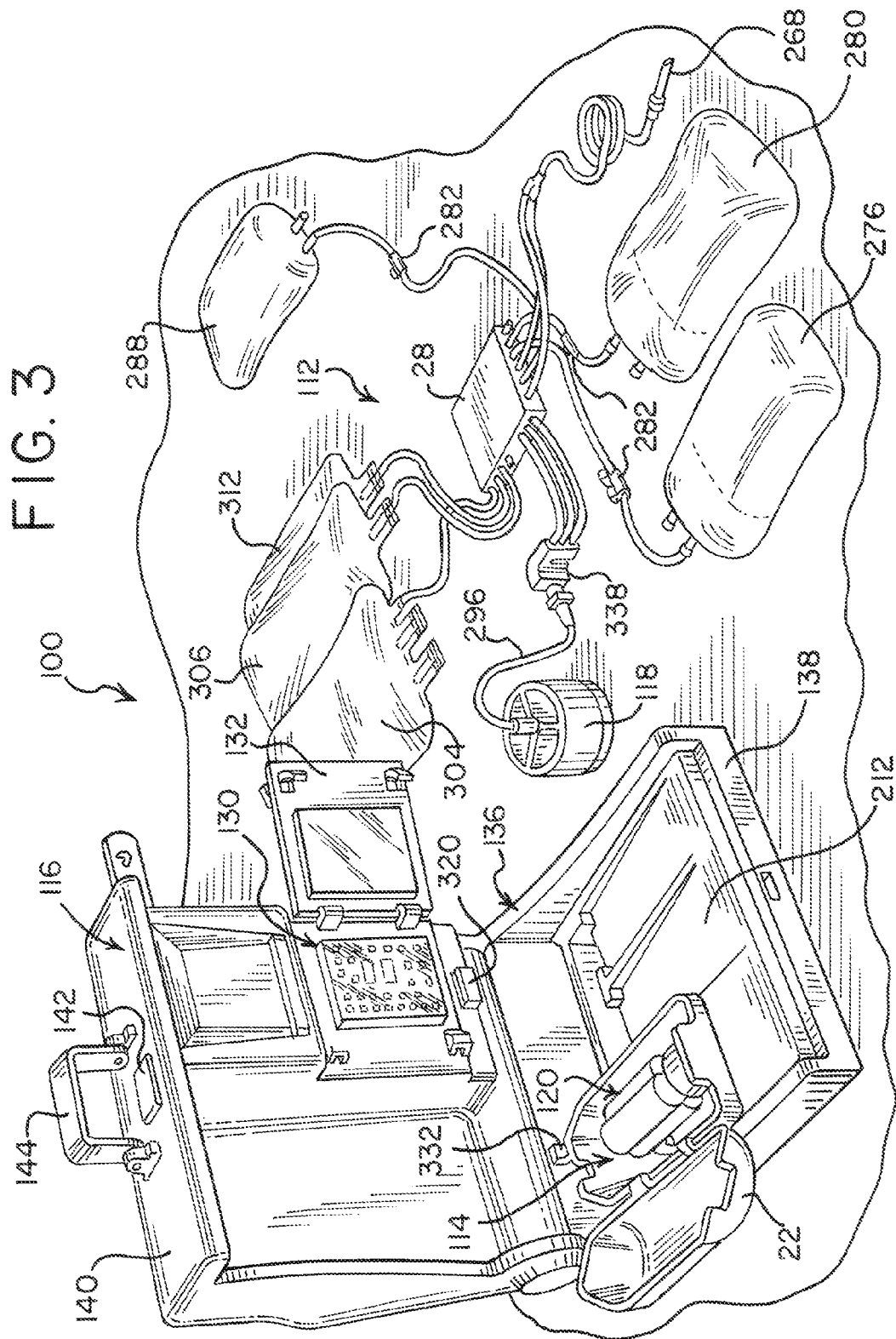


FIG. 4

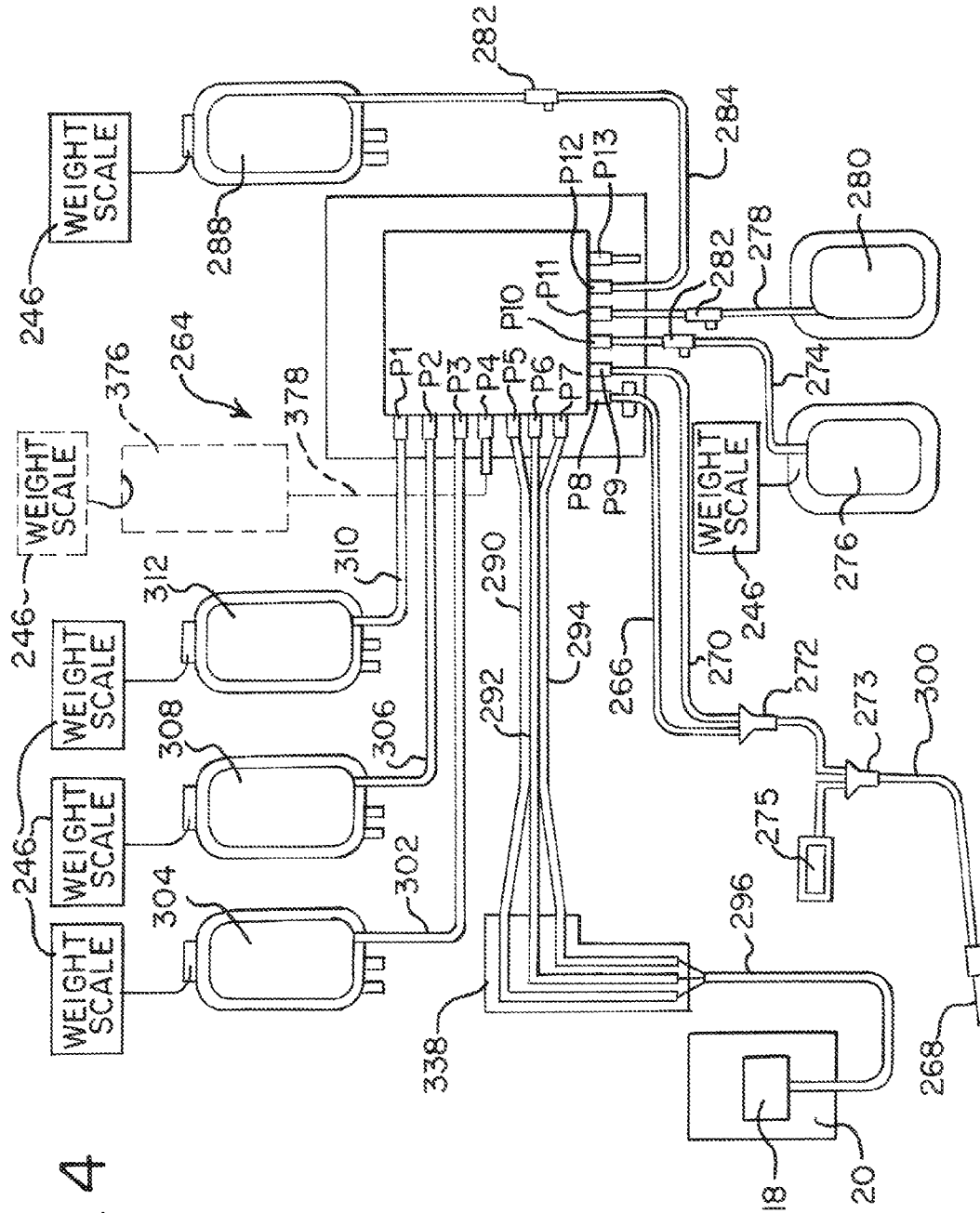


FIG. 5

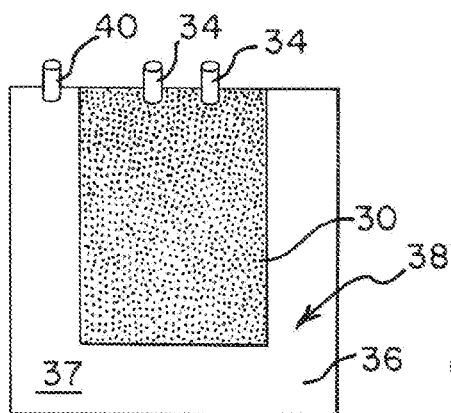


FIG. 6

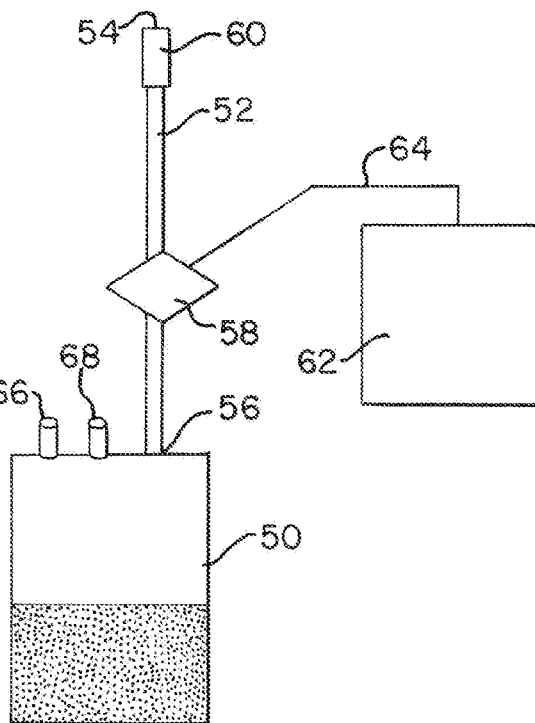
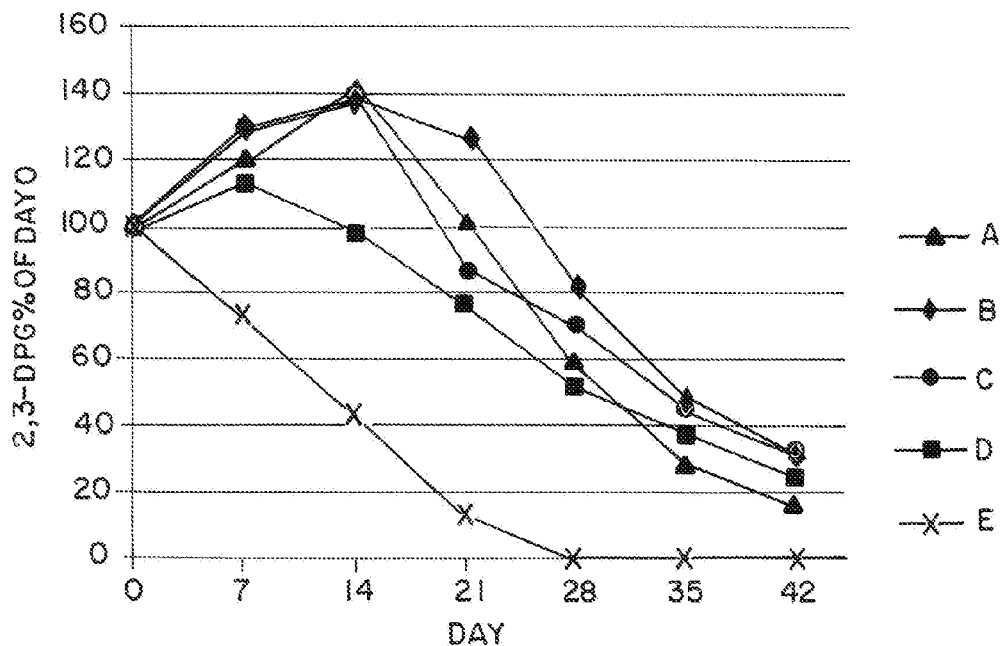


FIG. 7



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METHODS FOR STORING RED BLOOD CELL PRODUCTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is a divisional application of and claims priority to U.S. patent application Ser. No. 13/502,934, which is a National Stage, entered on Apr. 19, 2012, of PCT Application No. PCT/US2010/050036, which was filed on Sep. 23, 2010, which claims the benefit of U.S. Provisional Patent Application Ser. No. 61/254,550, filed Oct. 23, 2009, all of which are incorporated herein by reference in their entireties.

TECHNICAL FIELD

The subject matter of the disclosure that follows is generally directed to methods for providing readily transfusable, red blood cells with minimal plasma, to red blood cell products provided by such methods, and to systems for providing the same. More particularly, the disclosure that follows relates to methods and systems for providing readily transfusable, red blood cell compositions or products that include a red blood cell portion and a supernatant portion wherein the supernatant portion includes a reduced and selected volume of plasma (as a percentage of the supernatant volume).

BACKGROUND

The administration of blood and/or blood components is common in the treatment of patients suffering from disease or blood loss. Rather than infuse whole blood, it is more typical that individual components be administered to the patient in need. For example, administration (infusion) of platelets is often prescribed for cancer patients whose ability to make platelets has been compromised by chemotherapy. Red blood cells are typically administered to patients who have suffered a loss of blood, anemia or other disorders. Infusion of plasma may also be prescribed for therapeutic reasons and, more recently, the harvesting and administration of stem cells has received widespread interest within the medical community. Thus, it is often desirable to separate and collect a blood component, (e.g., red blood cells, platelets, plasma, stem cells) from whole blood and then treat the patient with the specific blood component. The remaining components may be returned to the donor or collected for other uses.

There are several factors to be considered in the separation, collection and storage of blood components for subsequent transfusion. For example, the presence of white blood cells (e.g., leukocytes) in a blood component collected for administration is often considered to be undesirable as such leukocytes can trigger an adverse response in the recipient-patient. As a result, blood components are often "leukoreduced" prior to transfusion. Also, the presence of certain antibodies in plasma has been correlated with the occurrence of TRALI (transfusion-related acute lung injury) in some patients receiving blood components such as red blood cells. Consequently, while plasma is present to some degree in transfusable red blood cells and platelets, it is desirable to reduce the amount of plasma in the red blood cell or platelet preparation.

Red blood cells are often stored for long periods of time prior to transfusion. In this case, the environment in which the cells are stored may have to be carefully controlled to optimize or at least maintain cell properties required for effective transfusion. For example, it is usually desirable to at least

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maintain adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) levels and limit hemolysis during storage.

Additive solutions useful for improving the storage environment of red blood cells are disclosed, for example, in pending U.S. application Ser. No. 12/408,483, filed Mar. 20, 2009 (Mayaudon et al.), and a continuation-in-part of the '483 application which is being filed on the same day as the present application and is entitled "Red Blood Cell Storage Medium for Extended Storage" (Mayaudon et al.), which serial number has not yet been assigned but which is identified by Applicant's reference number F-6542 CIP, which applications are incorporated by reference herein in their entireties.

Other additive solutions for improving the storage environment for red blood cells (and other components) are disclosed, for example, in U.S. Pat. No. 5,250,303 (Meryman), incorporated by reference herein, which discloses solutions for the extended storage of red blood cells. The solutions disclosed therein are generally chloride-free, hypotonic solutions which provide for the long-term storage of red blood cells. According to U.S. Pat. No. 5,250,303 to Meryman, the chloride-free, effectively hypotonic solutions induce a "chloride-shift" which, in turn, leads to a rise in the intracellular pH of the red blood cells. Also, according to Meryman, the rise in intracellular pH appeared to be correlated with rise in ATP and/or 2,3 DPG and, thus, prolonged storage times.

Although, Meryman recognized the benefit of using hypotonic solutions to extended storage of red blood cells generally, Meryman did not appreciate the benefits of using selected relative amounts of additive solution and plasma in the storage environment for the red cells, nor did Meryman describe systems for providing such red blood cell products.

Thus, there still exists a need for methods and systems that provide readily transfusable red blood cells whereby the red blood cell environment can be controlled and enhanced by preserving the red blood cells and reducing the potential of TRALI.

SUMMARY

In one aspect, the present disclosure is directed to a method for preparing transfusable and concentrated red blood cells with reduced waste products. The method includes (a) introducing concentrated red blood cells that have been separated from whole blood and an additive solution comprising glucose, a buffer, mannitol, adenine and a salt to stabilize said concentrated red blood cells into a multi-chambered container system. The multi-chambered container system includes an outer container including a pair of sealed walls defining an interior chamber of the outer container and an inner container housed within the interior chamber of the outer container. The inner container includes walls of a porous material defining an interior chamber of the inner container, the interior chamber of the inner container having a volume smaller than the volume of the interior chamber of the outer container. The porous material is selected to allow passage of only certain components or compounds across the wall but prevent the passage of red blood cells. A port communicates with the interior chamber of the outer container and at least one port communicates with the interior chamber of the inner container wherein (i) the concentrated red blood cells are introduced into the interior chamber of the inner container through the port communicating therewith and (ii) the nutrient containing additive solution is introduced into the interior chamber of the outer container through the port communicating therewith. The method further includes holding

the concentrated red blood cells within the interior chamber of the inner container of the container system for a selected period of time.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a flow chart setting forth the method of providing readily transfusable, red blood cells in accordance with the disclosure set forth herein;

FIG. 2 is a plan view of a processing set suitable for providing red blood cell product with minimal plasma in accordance with the method described herein;

FIG. 3 is a perspective view of an apheresis system suitable for practicing the methods described herein;

FIG. 4 is a plan view of a processing set for use with the system of FIG. 3;

FIG. 5 is a plan view of a container system suitable for use with the method described herein;

FIG. 6 is a plan view of another container system suitable for use with the method described herein; and

FIG. 7 is a graph showing extracellular 2,3-DPG levels during storage of red blood cell preparations made by different procedures.

DETAILED DESCRIPTION

The subject matter of the present disclosure relates generally to methods and systems for processing readily infusible red blood cells, systems useful for carrying out such methods, and the red blood cell products obtained by such methods and systems.

Methods of processing red blood cells and/or providing a red cell product that are/is readily transfusable are explained in detail below. By "readily transfusable," it is meant that the red blood cell preparation has a sufficiently reduced volume of plasma so as to minimize the potential incidence of TRALI, retains acceptable levels of ATP and 2,3-DPG, and acceptable levels of % hemolysis after storage. In addition, "readily transfusable" also refers to a volume of the red blood cell composition or product that can be safely infused into the patient.

As noted above, the presence of antibodies in plasma is correlated with the occurrence of TRALI. Accordingly, the systems and methods disclosed herein may be effective in reducing the incidence of TRALI. Thus, the plasma content in the supernatant of the red blood cell composition or product prepared using the methods of the present disclosure is no greater than about 15%. In a further embodiment, the plasma content may typically be from about 6% to about 11% of the supernatant volume of the red blood cell composition, or product.

As noted above, the red blood cell products disclosed herein are also considered readily transfusable because ATP and 2,3-DPG levels remain sufficiently elevated after extended storage. For example, red blood cell products and compositions of the type disclosed herein maintain acceptable levels of ATP for up to 42 days of storage. Also the stored red blood cells retain more than 50% of initial 2,3-DPG levels after 28 days of storage. More preferably, the stored red blood cells retain more than 80% of initial 2,3-DPG levels after 28 days of storage. Finally, red blood cell products and compositions of the type described herein maintain acceptable levels of % hemolysis after extended storage.

The systems and methods of the present disclosure may also be used to establish or control the conditions under which red blood cell products may be stored before transfusion. Thus, the methods may be used to introduce additive solu-

tions or reagents that may improve the storage properties of the red blood cells, thereby providing a transfusable red blood cell product.

In one embodiment, the method for providing readily transfusable, red cells disclosed herein (and depicted in FIG. 1) includes providing a quantity of blood, typically in a container. As used herein, the term "blood" is meant to include unfractionated and typically anticoagulated whole blood as well as a red blood cell concentrate that has been previously derived (i.e., separated) from whole blood. Thus, for example, the blood may be packed or concentrated red blood cells (i.e., red blood cell concentrate) having a hematocrit of at least 70% and more preferably at least 80% and most preferably at least 90% or higher.

Preferably, however, the "blood" is provided as whole blood in a container which can be a blood processing, collection or storage container of the type conventionally used in so-called "manual" or "whole blood" processing, or containers such as those used in automated apheresis. With regard to automated apheresis, the container in which the separation of blood or the processing of blood components may occur may also refer to the chamber of the separation device, such as a centrifuge or spinning membrane. Non-limiting examples of these are the separation chambers used in the Alyx® separator, Amicus® separator and Autopheresis®-C separator, all made and sold by Fenwal, Inc. of Lake Zurich, Ill. Regardless of whether the "blood" is provided as unfractionated whole blood or an already separated-from-whole blood red blood cell concentrate, the blood is separated (for the first time or further separated, depending on the composition of the starting "blood") into red blood cell concentrate and plasma, including any residual anticoagulant from the initial draw.

Where the source of blood is whole blood, a typical volume of whole blood is approximately 400-500 ml. In accordance with the method described herein, the blood may be spun to preferably result in a red blood cell concentrate having a hematocrit of preferably at least 90%. Plasma and residual anticoagulant form a supernatant layer, while the volume of the red blood cell concentrate separated from the supernatant may be approximately 150-250 ml. Once the desired volume of the packed red blood cells or red blood cell concentrate has been obtained, the method of the present disclosure includes physically separating and removing substantially all of the supernatant plasma layer (with anticoagulant) from the red blood cells. Not all of the supernatant, however, is removed and therefore the initial red blood cell concentrate will typically include about 10-30 ml of remaining supernatant.

As will be described in connection with systems used for carrying out the method disclosed herein, the separated plasma may be expressed to a satellite container of an integrated processing set. Plasma may be expressed using a component extraction device such as the Optipress, available from Fenwal, Inc. of Lake Zurich, Ill. or Compomat, available from Fresenius Kabi of Bad Homburg, Germany. The separated and now isolated plasma may be further processed, as necessary. The plasma may be "platelet-rich plasma" or "platelet-poor plasma" which in the case of the latter, may include a layer of buffy coat between the supernatant platelet-poor plasma layer and packed red blood cell layer.

With substantially all of the plasma (and anticoagulant) of the supernatant removed, a selected quantity or volume of additive solution is added to the initial red blood cell concentrate remaining in the initial container in which the whole blood (or other "blood") was provided, or in a separate container. The volume of additive solution added to the initial red blood cell concentrate may be anywhere between approximately 50-500 ml, with between about 100-300 ml being

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preferred and approximately 200 ml being most typical and preferred. The initial red blood cell concentrate that has been combined with the selected volume of additive solution is referred to herein as the "intermediate" blood cell product. The intermediate blood cell product preferably includes between approximately 150-250 ml, and more preferably approximately 200 ml of red blood cells, approximately 10-30 ml of the remaining supernatant and the added volume of additive solution as described above.

In accordance with one embodiment of the present disclosure, such intermediate red blood cell product may be further separated into a further red blood cell concentrate and a supernatant component that includes plasma, additive solution and anticoagulant. As described above, separation of the red blood cell concentrate from supernatant may be accomplished by centrifuging, by membrane separation or even by gravity.

Once the intermediate red blood cell product has been separated to provide a red cell component and a supernatant component, substantially all of the supernatant is expressed off into a satellite or other container, to provide a "treated" red blood cell concentrate. The treated red blood cell concentrate will typically include approximately 150-250 ml of red blood cells and a hematocrit of preferably greater than 80% and more preferably greater than 90%. The treated red blood cell concentrate may also include some remaining supernatant component having a volume of approximately 10-30 ml that includes plasma, additive solution and anticoagulant.

The "treated" red blood cell concentrate may then be combined with another selected (or second) amount of an additive solution of the type described above. For example, approximately 100-150 ml of additive solution may be added to the treated red blood cell concentrate, and more preferably, approximately 105-110 ml of additive may be added to the treated red blood cell concentrate to provide a final red blood cell product. Thus, upon addition of the additive solution to the treated red cell concentrate, the "final" red blood cell product or composition includes the treated red blood cell concentrate and the supernatant component made up of the residual plasma, anticoagulant and additive solution. The final and readily transfusable red blood cell product may include between approximately 150-250 ml of red blood cells, approximately 100-150 ml of additive solution and approximately 10-30 ml of the remaining supernatant component that includes plasma, additive solution and anticoagulant for a total volume of approximately 260-430 ml. This "final" product, which preferably will have a hematocrit of about 50-60% may then be stored until use or transfusion. In accordance with the method described herein, the percentage of plasma in the combined volume of additive (e.g., approximately 100-150 ml) and remaining supernatant component (e.g., approximately 10-30 ml) is no greater than 15%, and more preferably, may be between about 6% and 15%.

In a preferred embodiment, as mentioned above, the percentage amount of plasma as a percentage of the volume of additive solution combined with the volume of remaining supernatant component of the "final" red blood cell product is preferably no greater than about 15%. As will be described below, a supernatant component that includes no greater than 15%, provides an environment where the overall plasma volume is sufficiently low that the incidence of TRALI can be diminished and the levels of ATP and 2,3-DPG sustained such that the red blood cells are transfusable even after prolonged storage.

While the method described above contemplates more than one addition of the additive solution to arrive at the "final" product, further additions of additive solutions and removals

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of supernatant may be desired and required to arrive at a plasma level that is no greater than 15% of the supernatant. Alternatively, a single "wash" with an additive solution may also be sufficient. That is, a separation of "blood" into red blood cell concentrate and plasma, followed by one and only one addition of additive solution wherein the plasma makes up no greater than 15% of the supernatant is also within the scope of the invention described herein.

A variety of systems may be used to obtain a red blood cell product in accordance with the present disclosure. For example, FIG. 2 shows an embodiment of a system (processing set) 8 which may be employed to provide a red blood cell product according to the disclosure herein. In this embodiment, this system includes a plurality of interconnected containers in open or openable fluid communication with each other. The system or set may be closed or functionally closed without the need for sterile connection or any containers. A first bag 12 contains the blood to be processed. The containers and tubing defining the various flow paths may be made from a suitable plastic material of the type used in the medical field, such as, but not limited to polyvinyl chloride. The materials used for the containers and tubing and, thus, the entire set are/is sterilizable by, but not limited to, autoclaving.

As shown in FIG. 2, processing set 8 includes a plurality of plastic containers interconnected by tubing segments. Set 8 includes a first container 12 for holding the source of blood such as, but not limited to, whole blood to be processed in accordance with the method described herein. As will be described below, set 8 also includes satellite containers 14, 16, 18 and 20. Preferably, container 14, initially empty, will receive the final red blood cell product. A leukoreduction filter 22 may also be provided to leukoreduce the red blood cells. Containers 16 and 18 preferably contain an additive solution as described above, while container 20, also initially empty, will receive plasma separated from source blood in container 12. The container 12 is in fluid communication with each of four other bags (14, 16, 18, 20) through flow paths defined by tubing 21. Flow through processing set 8 may be controlled by flow controllers such as clamps, frangible connectors, valves or switches which are generally referenced by reference numeral 24.

Set 8, as shown in FIG. 2, also may include means for accessing the vascular system of a donor, such as a needle, blunt cannula or other access member, generally referenced by numeral 26, and a sample diversion and collection subunit 28 including sample pouch 30. These features are shown for illustrative purposes only as the method described herein and recited in the appended claims does not require and is not dependent upon connection to a living donor, just as it does not require administration of the final blood product to the same donor or a patient. Although the method of the present disclosure speaks to providing readily transfusable red blood cells, the later transfusion is not necessarily required as part of the method. In short, in its broader aspects, the method of the present disclosure is directed to the processing of blood regardless of its source and/or the timing of its collection, and/or the subsequent administration of it to a patient or donor.

Thus, according to one embodiment for providing a red blood cell product, blood such as, but not limited to whole blood, in container 12. As noted above, the volume of whole blood in container 12 is typically between 400-500 ml. The blood is subjected to a separation step such as centrifugation to separate the blood into red blood cell concentrate and plasma. After centrifugation, plasma may then be manually separated from the whole blood and transferred to a second container 20. The plasma in the second container 20 may be

retained for further processing. The red blood cell concentrate is retained in the first container **12** and includes about 200 ml of red cells and about 10-30 ml of plasma and anticoagulant (e.g., CPD).

After the plasma has been expressed from the container and physically separated from red blood cell concentrate (by, for example, an Optipress or other component extractor), a selected volume, approximately 200 ml, of an additive solution of the type described above may be transferred from a container **16** to container **12** containing the red blood cell concentrate to form an intermediate red blood cell product. After combining the additive solution with the red blood cell concentrate, the intermediate red blood cell product may be further processed by, for example, centrifuging the contents of at least container **12** and separating the supernatant, containing plasma and additive solution, from the red blood cell concentrate. (It will be appreciated that platelet-rich-plasma in container **20** may be simultaneously centrifuged to provide plasma and platelet concentrate). Preferably, the intermediate solution is subjected to a hard spin of at least 5,000 g for approximately 10 minutes such that the resulting red blood cell concentrate has a hematocrit of approximately 90%. As a consequence of mixing the additive solution with the red blood cell concentrate, separating the intermediate solution and expressing the supernatant, the plasma content of the red blood cell composition is reduced.

After the supernatant has been expressed or otherwise physically separated from the red blood cell concentrate, a second portion of a selected volume of an additive solution may be transferred from a container **18** to the red blood cell concentrate remaining in the first container **12**, to provide, preferably, the "final" product. The red cell concentrate remains in contact with the additive solution during the period of storage and until use. Where the volume of the red blood cell concentrate is approximately 150-250 ml, an additive solution of between about 100-150 ml may be used, and wherein about 105 ml of additive solution is preferred.

In an alternative embodiment, the initial step of separating whole blood into red blood cell concentrate and plasma may be omitted so that the additive solution (container **16**) may be directly added to the whole blood in container **12**. This is illustrated, for example, in FIG. 1, where the addition of additive solution to whole blood is shown as a phantom (dotted) lines/arrow. In other words, whole blood in the first container **12** is combined with a selected volume of an additive solution before the separation and expression of plasma from the first container. Containers may be sized so as to allow for the combination of whole blood and additive solution which may be provided in a volume of at least approximately 100 ml, preferably greater than 100 ml and, more preferably, about 100-350 ml. After combining the additive solution with the whole blood, the combination is centrifuged to provide a red blood cell concentrate and supernatant portion. The supernatant is then expressed into the container **20**, leaving a red blood cell concentrate in the container **12**. A further volume of additive solution may be added to the red blood cell concentrate, as required, as in the manner described above.

The method disclosed herein may also be practiced using automated apheresis devices. For example, as mentioned briefly above, the Alyx® separator, Amicus® separator and/or the Autopheresis-C® separator, all made by Fenwal Inc., of Lake Zurich Ill., USA are examples of devices that may be used to provide red blood cell products with reduced plasma in accordance with the method described herein.

In one example, FIG. 3 shows an embodiment of the Alyx® separation system that may be used to provide a red blood cell

preparation according to the present disclosure. An exemplary system is described in greater detail in U.S. Pat. No. 6,348,156, which is incorporated by reference herein in its entirety. The system is also briefly described below. Examples of the Autopheresis-C® and the Amicus® separators, which may also be used to provide red cell products or concentrates of the type described herein are described in U.S. Pat. No. 5,194,145 to Schoendorfer and U.S. Pat. No. 5,547,453 to DiPerna, respectively, which are also incorporated herein by reference in their entireties.

The system **100**, as shown in FIG. 3, includes three principal components. These are (i) a liquid and blood processing set **112**; (ii) a blood processing device **114** that interacts with the flow set **112** to cause separation and collection of one or more blood components; and (iii) a controller **116** that governs the interaction to perform a blood processing and collection procedure selected by the operator.

The blood processing device **114** and controller **116** are intended to be durable items capable of long term use. In the illustrated and preferred embodiment, the blood processing device **114** and controller **116** are mounted inside a portable housing or case **136**. The case **136** presents a compact footprint, suited for set up and operation upon a table top or other relatively small surface. The case **136** is also intended to be transported easily to a collection site.

The case **136** includes a base **138** and a hinged lid **140**, which opens (as FIG. 3 shows) and closes. The lid **140** includes a latch **142**, for releasably locking the lid **140** closed. The lid **140** also includes a handle **144**, which the operator can grasp for transporting the case **136** when the lid **140** is closed. In use, the base **138** is intended to rest in a generally horizontal support surface.

The case **136** can be formed into a desired configuration, e.g., by molding. The case **136** is preferably made from a lightweight, yet durable, plastic material.

The flow set **112** is intended to be a sterile, single use, disposable item. Before beginning a given blood processing and collection procedure, the operator loads various components of the processing set **112** in the case **136** in association with the device **114**. The controller **116** implements the procedure based upon preset protocols, taking into account other input from the operator. Upon completing the procedure, the operator removes the flow set **112** from association with the device **114**. The portion of the set **112** holding the collected blood component or components is removed from the case **136** and retained for storage, transfusion, or further processing. The remainder of the set **112** is removed from the case **136** and discarded.

The processing set **112** shown in FIG. 3 includes a blood processing chamber **118** designed for use in association with a centrifuge. Accordingly, as FIG. 2 shows, the processing device **114** includes a centrifuge station **120**, which receives the processing chamber **118** for use. The centrifuge station **120** comprises a compartment formed in the base **138**. The centrifuge station **120** includes a door **122**, which opens and closes the compartment. The door **122** opens to allow loading of the processing chamber **118**. The door **122** closes to enclose the processing chamber **118** during operation.

The centrifuge station **120** rotates the processing chamber **118**. When rotated, the processing chamber **118** centrifugally separates whole blood received from a donor into component parts, e.g., red blood cells, plasma, and buffy coat comprising platelets and leukocytes.

It should also be appreciated that the system **110** need not separate blood centrifugally. The system **110** can accommodate other types of blood separation devices, e.g., a membrane blood separation device.

As described in U.S. Pat. No. 6,348,156, system **100** includes a fluid pressure actuated cassette **128**. Cassette **128** interacts with pneumatic actuated pump and valve station **130**. The pump and valve station **130** apply positive and negative pneumatic pressure upon cassette **128** to direct flow through the system.

FIG. 4 schematically shows a processing set **264**, which, by selective programming of the blood processing circuit implemented by cassette **128**, is capable of performing the blood processing method described herein. The set **264** includes a donor tube **266**, which is attached (through y-connectors **272** and **273**) to tubing **300** having an attached phlebotomy needle **268**. The donor tube **266** is coupled to the port P8 of the cassette **128**.

A container **275** for collecting an in-line sample of blood drawn through the tube **300** is also attached through the y-connector **273**.

An anticoagulant tube **270** is coupled to the phlebotomy needle **268** via the y-connector **272**. The anticoagulant tube **270** is coupled to cassette port P9. A container **276** holding anticoagulant is coupled via a tube **274** to the cassette port P10. The anticoagulant tube **270** carries an external, manually operated in line clamp **282** of conventional construction.

A container **280** holding a red blood cell additive solution of the type described herein is coupled via a tube **278** to the cassette port P3. The tube **278** also carries an external, manually operated in line clamp **282**. A container **288** holding saline is coupled via a tube **284** to the cassette port P12.

FIG. 4 shows the fluid holding containers **276**, **280**, and **288** as being integrally attached during manufacture of the set **264**. Alternatively, all or some of the containers **276**, **280**, and **288** can be supplied separate from the set **264**. The containers **276**, **280**, and **288** may be coupled by conventional spike connectors, or the set **264** may be configured to accommodate the attachment of the separate container or containers at the time of use through a suitable sterile connection, to thereby maintain a sterile, closed blood processing environment. Alternatively, the tubes **274**, **278**, and **284** can carry an in-line sterilizing filter and a conventional spike connector for insertion into a container port at time of use, to thereby maintain a sterile, closed blood processing environment.

The set **264** further includes tubes **290**, **292**, **294**, which extend to an umbilicus **296**. When installed in the processing station, the umbilicus **296** links the rotating processing chamber **18** with the cassette **28** without need for rotating seals. The tubes **290**, **292**, and **294** are coupled, respectively, to the cassette ports P5, P6, and P7. The tube **290** conveys whole blood into the processing chamber **18**. The tube **292** conveys plasma from the processing chamber **18**. The tube **294** conveys red blood cells from processing chamber **18**.

A plasma collection container **304** is coupled by a tube **302** to the cassette port P3. The collection container **304** is intended, in use, to serve as a reservoir for plasma during processing. A red blood cell collection container **308** is coupled by a tube **306** to the cassette port P2. The collection container **308** is intended, in use, to receive a first unit of red blood cells for storage. A whole blood reservoir **312** is coupled by a tube **310** to the cassette port P1. The collection container **312** is intended, in use, to serve as a reservoir for whole blood during processing. It can also serve to receive a second unit of red blood cells for storage.

Using an automated apheresis device of the type shown in FIGS. 3 and 4, whole blood in container **312** is introduced into processing chamber **118** where it is separated into packed red blood cells and plasma. Packed red cells flow from processing chamber **118** to red blood cell container **308**. At least a portion of the red cells may be transferred from container **308** to

container **312** where they are combined with an additive solution of the type described above from container **280** to provide an intermediate red blood cell product. The intermediate red blood cell product may be introduced into processing chamber **118** to separate red blood cell concentrate from “supernatant” (i.e., plasma, additive solution and an anticoagulant such as ACD). The concentrated red blood cells may be returned to container **308** where they may be combined with another volume of additive solution from container **280**. The red blood cell concentrate may be combined with the additional additive before or during leukoreduction. The “final” red blood cell product may be transferred and stored in a final storage container (not shown).

As indicated above, it may not be necessary to process all of the red blood cell concentrate in container **308**. The system may be programmed to deliver only a portion of the red cell concentrate to, for example, container **312**, for combination with the additive solution, and for removal of supernatant such that the final red blood cell product will have a volume of plasma that is no greater than 15% of the supernatant volume in the final product. This “partial wash” reduces the time required for overall processing.

In an alternative embodiment, the system may also be programmed to deliver red blood cells and additive solution separately to the processing chamber **18** and thereby allow mixing of the cells and solution and formation of the intermediate product to occur within the chamber.

FIG. 5 shows a further embodiment that may be used to provide a red blood cell product with minimal plasma in accordance with the method disclosed herein. In this embodiment, a first container **30** includes one or more walls **32** made of a porous material to, in effect, provide a porous membrane. The container may have ports **34** for accessing the interior chamber of the container. Container **30** is enclosed and housed within second container **36**. The walls **37** of container **36** are non-porous and non-permeable. The volume of the second container is generally more than the volume of the first container. This container may also have ports **40** for accessing the interior of the container.

According to one embodiment, a red blood cell composition, is introduced into container **30** and an additive solution is introduced into the chamber **38** of the second container **36**. As a percentage of the plasma, additive solution and anticoagulant contained within the container system of FIG. 5, the volume of plasma relative to such components (other than the red blood cells—i.e., supernatant) is preferably no greater than 15%. In this example, the term “supernatant” is not used to describe a discrete and separate layer, but rather that portion of the overall composition that is not red cell concentrate. The porosity of the wall **32** of container **30** is selected such that selected components (but not the red blood cells themselves) are permitted to move, by diffusion, from one container to another. For example, molecules of the additive solution may move from the second container to the first container, thereby stabilizing the red blood cells during storage. More particularly, during storage, ions and other small molecules are able to diffuse back and forth across the porous membrane of container **30**. Thus, the concentration of chloride ions and any other deleterious small molecules (cytokines, free Hb) in the red blood cells will be reduced as they diffuse across the membrane. As a further added step, the concentrated red blood cells may be pre-diluted with the additive solution. This may minimize water movement across the membrane, thereby helping maintain the desired hematocrit. The red blood cells may be stored up to 42 days. In this way, a red blood cell product that has reduced plasma content may be formed.

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FIG. 6 shows a further embodiment of a system that may be used to provide a red blood cell product with minimal plasma. This embodiment includes a first container 50. The system also includes a vent line 52 that is inserted into the first container, the vent line 52 having distal end 54 and proximal end 56. The volume of the vent line is preferably from about 20 ml to about 50 ml. The vent line 52 may also include a flow control device 58 for controlling flow between containers 50 and 62 (discussed below). In some embodiments, the distal end of the vent line may be covered with or otherwise include a hydrophobic membrane 60. A second container 62 is in fluid communication with both the first container 50 and the vent line 52. In a preferred embodiment, the second container directly communicates with the vent line through tubing 64.

In this embodiment, the vent line is displayed as tubing, but in other embodiments it may be a bag or pouch.

According to one embodiment, a red blood cell composition may be introduced into the first container 50. The red blood cell composition may, for example, be whole blood. The composition may be separated by centrifugation into red blood cell concentrate, plasma and an intermediate layer of a "buffy coat" between the red blood cell concentrate layer and plasma (e.g., platelet poor). Using a conventional component extraction device, plasma may be expressed into the second container, leaving concentrated red blood cells in the first container. After the expression of plasma, a portion of red blood cells at the top of the concentrated red blood cell layer may be expressed into the vent line 52, to thereby ensure that substantially all of the plasma has been expressed from the container and thus further reducing the content of plasma of the concentrated red blood cells. In one embodiment, this portion of red blood cells is from about 20 to 50 ml per unit of whole blood, which corresponds to the volume of the flow path within vent line 52. While some red blood cells may be removed to container 50, removal of substantially all of the plasma occurs.

A mechanical flow control device 58 may be used to control the flow of plasma and of red blood cells from the first container. For example, in one embodiment, a rotating cam may be used to "pinch off" the plasma or red blood cell flow paths. An operator may release a lever to allow plasma to flow into the first container. When the flow of plasma is complete, the operator closes the plasma flow path and causes the flow of red blood cells to the vent line to begin.

The vent line automatically stops filling when the vent line is full. The presence of hydrophobic membrane 62 at the distal end 54 of vent line 52 may facilitate the arrest of red blood cell flow. The operator may then seal first container 50 from the second container 62 and vent line 52 and an additive solution may be added to container 50 through ports 66 and 68 for storage. The volume of plasma as a percentage of the supernatant is preferably no greater than 15%.

According to this embodiment, an additive solution may be added to the concentrated red blood cells and the cells stored for subsequent transfusion.

Additive Solution

As mentioned above, there are many additive solutions known for the conditioning, treatment and/or preservation of red blood cells. However, solutions that are at least substantially chloride-free, nutrient-containing, having a pH that is neutral or above-neutral (e.g., preferably 7.0 or greater, and more preferably 8.0 or greater) are particularly well suited for the methods disclosed herein.

In one embodiment, in accordance with the present disclosure, the red blood cells are treated with an additive solution

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that is substantially chloride-free. Chloride-free additive solutions are described in co-pending application Ser. No. 12/408,483, filed Mar. 20, 2009, and a continuation-in-part of the '483 application entitled "Red Blood Cell Storage Medium for Extended Storage" (Mayaudon et al.) which is being filed on the same day as the present application, which serial number has not yet been assigned but which is identified by Applicant's reference F-6542 CIP, the contents of which applications are hereby incorporated by reference in their entireties. The compositions of such additive solutions are also described in Table 1:

TABLE 1

Component (mmol/L)	E-Sol 2	E-Sol 3	E-Sol 4	E-Sol 5
Sodium Citrate dihydrate, EP	20.8	31.2	28.4	25.0
Sodium Phosphate dibasic, anhydrous	16.7	25.0	22.7	20.4
Mannitol, EP	33.3	50.0	45.4	39.9
Adenine, EP	1.4	2.0	1.9	2.0
Dextrose, monohydrate, EP	37.8	56.8	51.6	111
	150 ml	100 ml	110 ml	105 ml

Table 1 sets forth four exemplary formulations of storage solution suitable for addition to the concentrated (packed) red blood cells prepared from one unit of blood. The storage solutions are useful for the extended storage of red blood cells (e.g., approximately 42 days or greater) that have been separated from whole blood. The solutions disclosed herein are particularly useful for the extended storage of red blood cells that have been separated from whole blood that has been held for periods, such as 4 hours, 8 hours or more than 8 hours, including up to 26 hours.

As set forth in Table 1, the solution "E-Sol 2" is provided in a volume of 150 ml, "E-Sol 3" is provided in a volume of 100 ml, "E-Sol 4" is provided in a volume of 110 ml, and "E-Sol 5" is provided in a volume of 105 ml.

The storage solutions are generally applicable to the storage of red blood cells that have been manually separated from whole blood or have been separated using automated collection devices such as the Autopheresis-C® separator, the Amicus® separator and/or the Alyx® separator mentioned above.

In one embodiment, a red blood cell storage medium is provided that includes nutrients, buffers and salts. The red blood cell storage solution may be an aqueous solution which may include about 3 mM to about 90 mM sodium citrate; about 15 mM to about 40 mM sodium phosphate; about 20 mM to about 140 mM dextrose; about 20 mM to about 110 mM mannitol; and about 1 mM to about 2.5 mM adenine; the storage solution may have a pH of from about 8.0 to about 9.0. The osmolarity of the solution may be from about 200 mOsm/l to about 320 mOsm/l. The storage solution may optionally contain from about 10 mM to about 100 mM acetate. Optionally, guanosine may also be present in the storage solution from about 0.1 mM to about 2 mM. In addition, gluconate may be present from about 0 mM to about 40 mM. Optionally, a combination of acetate and gluconate may be used.

In another embodiment, an aqueous red blood cell storage medium is provided that may include about 3 to 90 mM of sodium citrate; 15 to 40 mM of sodium phosphate; 20 to 140 mM of glucose; 20 to 100 mM of mannitol and 1 to 2.5 mM of adenine. The storage solution may have a pH of from about 8.0 to about 8.8, and more preferably, about 8.4. The osmolarity may be in the range of about 150 to 350 mOsm L⁻¹ and more preferably, about 314 mOsm L⁻¹.

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In one example, the "E-Sol 5" storage medium identified in Table 1, above, is an aqueous solution that is constituted from a dextrose (i.e., glucose) solution that, at approximately 25° C. has a generally acidic pH in the range of 3.5 to 5.5 and more particularly a pH of 3.7 to 5.3, and even more preferably about 4.0, and a second solution that includes sodium phosphate, mannitol and adenine that has a generally basic pH in the range of 8.0 to 9.0. Once the two solutions are combined, the final E-Sol 5 composition includes the following components indicated, in the range of 3 to 90 mM of sodium citrate; 15 to 40 mM of sodium phosphate; 20 to 140 mM of glucose; 20 to 100 mM of mannitol and 1 to 2.5 mM of adenine, and more particularly, the components indicated in the amount shown in Table 1, above.

By way of example, but not limitation, illustrations of methods and systems for providing red blood cell products with reduced plasma as described herein are provided below:

Example 1

In this example, whole blood was processed using several different procedures to produce a red blood cell product. The red blood cell product produced by each procedure was stored for up to 42 days in the E-Sol 4 additive solution and cell properties were assayed at 7 day intervals.

FIG. 7 is a graph showing extracellular 2,3-DPG concentrations over time for the red blood cell products made by each procedure. Table 2 provides a key to the legend of FIG. 7.

TABLE 2

Sample	Procedure	% Plasma Content in Supernatant
A	Treat/Spin	7
B	Storage in excess additive solution	11
C	Alternate buffy coat	11
D	OptiPressII	15
E	Alyx Standard	29

For sample A in FIG. 7, 500 ml of whole blood was collected in a bag (Fenwal 4R1590 blood packs) and subjected to spin (5000 g) to separate plasma and red blood cells. Plasma was expressed from the bag by manual pressing. Approximately 220 ml of E-Sol 4 was transferred to the red blood cell concentrate and mixed forming an intermediate solution. The bag was centrifuged and the supernatant of plasma-containing solution expressed. 110 ml of E-Sol 4 was added to the concentrated red blood cells and the cells were stored and samples taken at seven day intervals for biochemical assays. The plasma content at each step of the procedure was determined as follows:

Calculation of Initial Plasma Volume

$$\text{Volume}_{\text{wB}}(\text{ml}) = \frac{\text{Weight}_{\text{BPU+WB}}(\text{g}) - \text{Tare Weight}_{\text{BPU}}(\text{g}) - 70 \text{ g CPD}}{\frac{\text{Hct}}{100} \times 1.09 \frac{\text{g}}{\text{mL}} + \left(1 - \frac{\text{Hct}}{100}\right) \times 1.03 \frac{\text{g}}{\text{mL}}}$$

$$[(\text{Volume}) \downarrow (\text{initial plasma}) (\text{ml})] = [(\text{Volume}) \downarrow \text{WB}(\text{ml}) \times (1 - \text{Hct}/100)]$$

Calculation of Plasma Volume after 1st Expression

$$\text{Volume}_{\text{expressed plasma}}(\text{ml}) = \frac{\text{Weight}_{\text{Bag5+plasma}}(\text{g}) - \text{Tare Weight}_{\text{Bag5}}(\text{g})}{1.03 \frac{\text{g}}{\text{mL}}}$$

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-continued

$$\text{Volume}_{\text{plasma remaining}}(\text{ml}) =$$

$$\text{Volume}_{\text{initial plasma}}(\text{ml}) - \text{Volume}_{\text{expressed plasma}}(\text{ml})$$

Calculation of % Plasma in Supernatant after Addition of E-Sol 4

$$\text{Volume}_{\text{AS added}}(\text{ml}) = \frac{\text{Weight}_{\text{Bag3+AS}}(\text{g}) - \text{Tare Weight}_{\text{Bag3}}(\text{g})}{1.01 \frac{\text{g}}{\text{mL}}}$$

$$\% \text{ Plasma}_{\text{supernatant}} = \frac{\text{Volume}_{\text{plasma remaining}}(\text{ml})}{\text{Volume}_{\text{plasma remaining}}(\text{ml}) + \text{Volume}_{\text{AS added}}(\text{ml})} \times 100\%$$

Calculation of Plasma Volume Remaining after 2nd Expression

$$\text{Volume}_{\text{plasma-AS expressed}}(\text{ml}) =$$

$$\frac{\text{Weight}_{\text{Bag3+plasma-AS}}(\text{g}) - \text{Tare Weight}_{\text{Bag3}}(\text{g})}{\frac{\% \text{ plasma}}{100} \times 1.03 \frac{\text{g}}{\text{mL}} + \left(1 - \frac{\% \text{ plasma}}{100}\right) \times 1.01 \frac{\text{g}}{\text{mL}}}$$

$$\text{Volume}_{\text{plasma-AS remaining}}(\text{ml}) = \text{Volume}_{\text{plasma remaining}}(\text{ml}) +$$

$$\text{Volume}_{\text{AS added}}(\text{ml}) - \text{Volume}_{\text{plasma-AS expressed}}(\text{ml})$$

$$\text{Volume}_{\text{residual plasma}}(\text{ml}) =$$

$$\text{Volume}_{\text{plasma-AS remaining}}(\text{ml}) \times \% \text{ Plasma}_{\text{supernatant}}$$

For sample B, a red blood cell concentrate was prepared from one unit of whole blood using a standard procedure for the Fenwal Alyx® device, as generally shown in FIGS. 3 and 4. An excess of additive solution, 330 ml of E-Sol 4, was mixed with the red blood cell concentrate in a bag and the preparation stored. In this case, the hematocrit during storage was approximately 30%. Samples for biochemical assays taken at 7 day intervals.

For sample C, a red blood cell product was prepared using a system similar to that shown in FIG. 6. Whole blood was subjected to centrifugation (5000 g for 10 min.) and the plasma expressed into an additional container. In addition, approximately 25 ml of red blood cells was expressed into a third container. 130 ml of E-sol 4 was added to the concentrated red blood cells and the cells were stored and samples taken at seven day intervals for biochemical assays. The results of the measured values for individual samples for 2,3-DPG values are summarized in Table 3. The data are also summarized in FIG. 7.

TABLE 3

Sample	Concentration of 2,3-DPG (μmol/ml)						
	0	7	14	21	28	35	42
A	2.5	3.3	3.6	1.5	1.0	0.6	0.4
B	1.8	2.4	2.7	2.3	1.9	1.6	1.2
C	2.2	2.5	2.9	1.9	1.6	1.0	0.8
D	2.4	3.3	3.5	2.6	1.9	1.3	1.0
E	2.1	2.7	2.5	1.1	1.3	0.4	0.2

For sample D, whole blood was subjected to a hard spin (5000 g for 10 min.) and plasma and a buffy coat layer were

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expressed using an Opti-Press II. Approximately 110 ml of E-Sol 4 was added to the concentrated red blood cells and the cells were stored and assayed for various biochemical parameters at 7 day intervals.

For sample E, a concentrated red blood cell sample was prepared using a standard Alyx® procedure from one unit of blood without further treatment with additive solution. Thus, this procedure serves as a control for Sample B.

Example 2

Fifteen whole blood (WB) units (500 ml±10%) were collected into 4R1590 blood packs (Fenwal) and stored for 1-4 hours at room temperature before separation (5000×g, 10 min). In twelve WB units, the plasma was expressed and the concentrated RBCs were washed with E-Sol 4 (220 ml) and separated again (5000×g, 10 min). E-Sol 4 (110 ml) was added to the RBC concentrate and subsequently leukofiltered (test). In the three remaining WB units, 110 ml of Adsol® (a chloride containing additive solution used as a control) was added to each and then leukofiltered. The RBC concentrates were stored for 42 days with weekly sampling of in vitro parameters. Summary data collected at day 0, 14, 21, and 42 are given in the table below (mean±SD). Residual plasma was 9.8±0.9 ml in test and 33.8±1.3 ml in control units. Extracellular pH (22° C.) and K⁺ (mmol/L) were similar at Day 42 for test and control (6.22±0.06 vs. 6.24±0.01) and (56.8±2.4 vs. 56.6±3.9), respectively. Glucose (mmol/L) was present throughout storage for test and control units (17.3±1.8 and 36.2±3.1, at Day 42, respectively). Lactate concentration (mmol/L) was higher in test units at Day 42 (37.6±2.5 vs. 31.8±1.8).

The results (table 4) show that at Day 42, RBCs stored with minimal residual plasma and E-Sol 4 show lower % hemolysis than non-washed controls. Notably, 2,3-DPG levels are at or above Day 0 levels for 21 days and ATP is maintained above 80% of Day 0 throughout 42-day storage.

TABLE 4

	Day 0	Day 14	Day 21	Day 42
% Hemolysis				
Test	0.08 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.02
Control	0.04 ± 0.01	0.08 ± 0.01	0.11 ± 0.02	0.23 ± 0.08
ATP, μmol/g Hb				
Test	4.2 ± 0.4	5.2 ± 0.5	4.9 ± 0.5	3.3 ± 0.4
Control	4.8 ± 0.5	5.0 ± 0.2	4.3 ± 0.9	3.0 ± 0.3
2,3-DPG, μmol/g Hb				
Test	14.4 ± 1.6	20.4 ± 2.1	14.4 ± 1.9	2.3 ± 1.0
Control	13.0 ± 2.0	4.1 ± 6.3	2.7 ± 2.3	0.3 ± 0.3

It will be understood that the embodiments described above are illustrative of some of the applications of the principles of the present subject matter. Numerous modifications may be made by those skilled in the art without departing from the spirit and scope of the claimed subject matter, including those combinations of features that are individually disclosed or claimed herein. For these reasons, the scope hereof is not limited to the above description but is as set forth in the following claims.

The invention claimed is:

1. A method for preparing transfusable and concentrated red blood cells with reduced waste products, the method comprising:

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(a) introducing concentrated red blood cells that have been separated from whole blood and an additive solution comprising glucose, a buffer, mannitol adenine and a salt to stabilize said concentrated red blood cells into a multi-chambered container system, said multi-chambered container system including an outer container comprising a pair of sealed walls defining an interior chamber of said outer container, an inner container housed within said interior chamber of said outer container, said inner container comprising walls of a porous material defining an interior chamber of said inner container, said interior chamber of said inner container having a volume smaller than the volume of said interior chamber of said outer container, said porous material selected to allow passage of only certain components or compounds across said wall but prevent the passage of red blood cells, a port communicating with said interior chamber of said outer container and at least one port communicating with said interior chamber of said inner container wherein:

- (i) said concentrated red blood cells are introduced into the interior chamber of said inner container through the port communicating therewith;
 - (ii) said nutrient containing additive solution is introduced into the interior chamber of said outer container through the port communicating therewith; and
- b) holding said concentrated red blood cells within the interior chamber of said inner container of said container system for a selected period of time.

2. The method of claim 1 wherein said additive solution is selected for prolonging the storage shelf life of said red blood cells.

3. The method of claim 1 wherein said additive solution comprises about 20 to about 140 mM of glucose, about 20 to about 100 mM mannitol, about 15 to about 40 mM sodium phosphate dibasic, about 3 to about 90 mM sodium citrate and about 1 to 2.5 mM adenine and has a pH of greater than 8.0.

4. The method of claim 1 wherein said porous material of said inner container has a porosity selected to allow passage of compounds and ions of said additive solution across said porous material.

5. The method of claim 1 wherein said porous material of said inner container has a porosity selected to allow passage of plasma between said inner container to said interior chamber of said outer container.

6. The method of claim 1 further comprising reducing the concentration of small molecules in said red blood cells.

7. The method of claim 6 further comprising reducing the concentration of one or more compounds selected from the group of cytokines, chloride ions and free hemoglobin.

8. The method of claim 1 comprising diluting said concentrated red blood cells before introducing said concentrated red blood cells into said container system.

9. The method of claim 1 wherein said selected period of time is at least 42 days.

10. The method of claim 1 wherein as a percentage of plasma, additive solution and anticoagulant within the container system, no more than approximately 15% comprises plasma.

11. The method of claim 1 further comprising removing the contents of the interior chamber of said outer container through an access port.

12. The method of claim 1 further comprising removing said concentrated red blood cells from said inner container

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through an access port that communicates with the interior chamber of said inner container after said selected period of time.

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